Tzu Chi Medical Journal 26 (2014) 119–122

Contents lists available at ScienceDirect

Tzu Chi Medical Journal

journal homepage: www.tzuchimedjnl.com

Original Article

Dose-dependent effects of isoflurane on cardiovascular function in rats

Chiu-Fen Yang [a, b, †], Michael Yu-Chih Chen [a, b, †], Tsung-I Chen [c], Ching-Feng Cheng [d, e, *]

[a] Department of Cardiology, Buddhist Tzu Chi General Hospital, Hualien, Taiwan
[b] Institute of Medical Science, Tzu Chi University, Hualien, Taiwan
[c] Center of Physical Education, Tzu Chi University, Hualien, Taiwan
[d] Department of Pediatrics, Tzu Chi University, Hualien, Taiwan
[e] Department of Medical Research, Buddhist Tzu Chi General Hospital, Hualien, Taiwan

ARTICLE INFO

Article history:
Received 28 April 2014
Received in revised form
10 July 2014
Accepted 22 July 2014

Keywords:
Anesthesia
Isoflurane
Pressure–volume loop
Ventricular function

ABSTRACT

Objectives: Isoflurane is a widely used anesthetic in clinical practice and animal experiments. It exerts cardioprotective effects by pre- and postconditioning but also has dose-dependent cardiovascular side effects. In this article, we aim to characterize the hemodynamic effects of isoflurane.
Materials and methods: We used a pressure–volume catheter to evaluate the hemodynamic changes in adult Sprague–Dawley rats ($n = 6$) given increasing concentrations of inhaled isoflurane anesthesia. The concentration was started at 0.5% (baseline) and gradually increased. Data on cardiovascular variables were recorded at each concentration.
Results: Heart rate, blood pressure, and left ventricular systolic and diastolic function decreased as isoflurane concentration increased. At a concentration of 3%, isoflurane significantly decreased myocardial contractility, blood pressure, and heart rate, and impaired left ventricular diastolic function.
Conclusion: High-dose isoflurane resulted in unfavorable hemodynamics. Old age and dehydration may predispose animals to the unfavorable hemodynamic effects of isoflurane. Determining the optimal isoflurane concentration for anesthesia or preconditioning is important. The effects of isoflurane anesthesia on aged and/or volume-depleted animals should be further investigated.

1. Introduction

Isoflurane is a common inhalational anesthetic in animal experiments and clinical practice. Thus, the cardiovascular characteristics of this agent should be carefully investigated. Isoflurane affects several hemodynamic parameters, which result in various cardiovascular side effects. It alters cardiac electrophysiological function and may cause arrhythmia and decrease cardiac contractility [1,2]. Furthermore, isoflurane decreases peripheral vascular resistance [3], leading to hypotension. Because it is a potent coronary vasodilator, isoflurane may, under certain conditions, cause a coronary steal phenomenon in ischemic regions [4]. However, isoflurane also has a dose-dependent cardioprotective effect. It can protect the heart against ischemia–reperfusion injury and may limit myocardial infarct size to an extent similar to that seen in ischemic preconditioning.

The cardioprotective mechanisms of volatile anesthetics have been extensively studied [5], but the mechanisms responsible for cardioprotection are not well understood. Phosphatidylinositol 3-kinase has a role in myocardial protection from both anesthetic and ischemic preconditioning [6]. Isoflurane has been used during cardiac surgery to exert a preconditioning protective effect [7]. However, the optimal isoflurane concentration for preconditioning may have undesirable hemodynamic effects on patients. Therefore, the effect of dose dependency on hemodynamics during isoflurane anesthesia should be investigated.

Pressure–volume analysis is a useful approach for examining *in vivo* intact ventricular function in all loading conditions. The reliability and reproducibility of pressure–volume catheters for simultaneous, real-time measurement of left ventricular (LV) pressure and volume make them useful for evaluating LV function

Conflicts of interest: none.

* Corresponding author. Department of Pediatric, Buddhist Tzu Chi General Hospital, 707, Section 3, Chung-Yang Road, Hualien, Taiwan. Tel.: +886 3 8561825x2227; fax: +886 3 8561825x3491.
E-mail address: chengcf@mail.tcu.edu.tw (C.-F. Cheng).

† These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.tcmj.2014.07.005

[8]. This technique has been widely used in humans and large animal studies since the mid-1980s. Recent advances in the development and validation of microconductance pressure–volume catheters have made it possible to use this approach to assess cardiac and pharmacological interventions in small animals. A technique for LV pressure–volume analysis in rats was recently introduced. However, only limited normative data are available [9].

We used a pressure–volume loop system to investigate the hemodynamic effects of different concentrations of inhalational isoflurane anesthesia in adult rats.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats ($n = 6$; age 8–12 months; weight, about 600 g) were purchased from BioLASCO Co., Ltd. (Taipei, Taiwan). The rats were kept in cages (2 rats per cage) in our laboratory animal center and fed with standard laboratory diet and tap water *ad libitum* on a 12-h/12-h light/dark cycle. All study procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Tzu Chi University, Hualien, Taiwan.

2.2. Experimental protocol

Anesthesia induction was performed with a mixture of 5% isoflurane and oxygen in an induction chamber. After induction, the animals were placed on a heating pad in the supine position. A tracheostomy was performed, and the animals were ventilated with a mixture of 1.5% isoflurane and oxygen by a Harvard rodent ventilator (Model 683; Harvard Apparatus, Holliston, MA, USA) during the instrumentation procedure. The ventilator was set at a tidal volume of $6.2 \times M^{1.01}$ (where M denotes the animal mass in kg) and a frequency of 70 ventilations/minute. The right carotid artery was exposed, and a pressure–volume catheter (SPR-901; Millar Instruments, Inc., Houston, TX, USA) was inserted into it and advanced into the LV while being guided by real-time pressure and volume signals. A polyethylene tube was inserted into the left jugular vein for administration of fluids. The signals were continuously recorded by a PowerLab data acquisition system and LabChart software (AD Instruments, Mountain View, CA, USA). Then, four different isoflurane concentrations—0.5% (baseline), 1%, 2%, and 3%—were given as the interventions. The experiment started at an isoflurane concentration of 0.5%, and the concentration was increased every 3 minutes. Heart rate, blood pressure, maximum first derivative of LV pressure over time (dP/dt max), and minimum first derivative of LV pressure over time (dP/dt min) were recorded continuously at each concentration and compared with values obtained at other concentrations. Inferior vena cava compression was performed at every isoflurane concentration, and the end-systolic pressure–volume relation (ESPVR) was recorded.

2.3. Statistical analysis

Descriptive statistics are expressed as means ± standard deviations for all hemodynamic variables. The Friedman test was used to evaluate the effects of different concentrations. A p value of < 0.05 indicated statistical significance. If the result was significant, the Dunn *post hoc* test was used to compare the difference between the value at baseline and the value at a given isoflurane concentration.

3. Results

3.1. Heart rate

Hemodynamic characteristics, including heart rate, blood pressure, and dP/dt, are shown according to isoflurane concentration in Table 1. The heart rate increased when the isoflurane concentration was increased from 0.5% to 1% and then progressively decreased with increasing isoflurane concentration. The difference in heart rate at a concentration of 1.5% (standard) versus 0.5% isoflurane (baseline) was not significant. However, heart rate significantly differed between the baseline concentration and the concentration of 3% (high dose; Fig. 1).

3.2. Blood pressure

Systolic blood pressure, diastolic blood pressure, and mean arterial pressure increased as the isoflurane concentration was increased from 0.5% to 1% and then progressively decreased with increasing isoflurane concentration. As compared with baseline, these variables were significantly lower at a concentration of 3%. However, LV end-diastolic pressure did not significantly change in relation to isoflurane concentration. The difference between diastolic aortic pressure and LV end-diastolic pressure (DBP − Ped), which represents coronary perfusion pressure, was also significantly lower than the baseline value at a concentration of 3% ($p < 0.001$).

3.3. Cardiac systolic function

At a concentration of 3%, dP/dt max, the maximum rate of pressure change in the ventricle, was significantly lower than at baseline. However, dP/dt max is load-dependent and, therefore, a

Table 1
Cardiovascular characteristics of rats according to the concentration of isoflurane anesthesia.

	ISO 0.5% (baseline)	ISO 1%	ISO 1.5%	ISO 2%	ISO 3%
Heart rate (bpm)	325.0 (16.9)	341.0 (29.1)	321.0 (23.4)	278.2 (31.7)	234.8 (21.8)*
SBP (mmHg)	154.2 (13.3)	160.0 (26.4)	126.2 (27.3)	100.4 (25.6)	85.0 (16.1)*
DBP (mmHg)	118.4 (9.7)	120.0 (24.2)	92.6 (33.1)	62.5 (30.1)	49.4 (23.1)*
MAP (mmHg)	130.3 (10.7)	133.3 (24.9)	103.8 (31.1)	75.1 (28.3)	61.3 (20.6)*
DBP − Ped (mmHg)	113.1 (10.0)	114.4 (24.1)	86.8 (33.6)	56.8 (29.8)	42.9 (22.3)*
Pes (mmHg)	154.5 (14.7)	158.9 (28.5)	127.8 (27.4)	103.9 (27.9)	86.0 (14.6)*
Ped (mmHg)	5.3 (2.0)	5.6 (1.4)	5.8 (1.6)	5.7 (2.0)	6.6 (2.8)
dP/dt max (mmHg/s)	10,278.2 (1278.6)	10,321 (717.7)	8424.5 (1784.9)	5782.8 (2249.1)	4312.5 (1315.9)*
dP/dt min (mmHg/s)	8009.5 (392.0)	7988.5 (907.8)	6537.3 (1757.2)	4736.0 (1911.8)	3663.2 (1200.6)*

Data are presented as mean (SD).
*$p < 0.05$ versus ISO 0.5% (baseline).
DBP = diastolic blood pressure; ISO = isoflurane; MAP = mean arterial pressure; Ped = left ventricular end-diastolic pressure; Pes = left ventricular end-systolic pressure; SBP = systolic blood pressure.

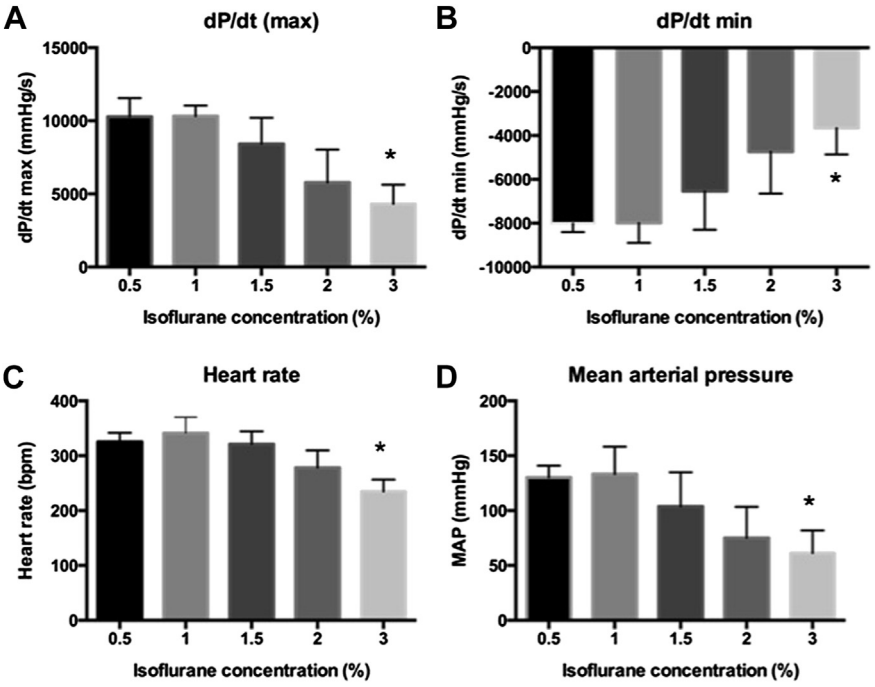

Fig. 1. Effects of different concentrations of isoflurane on hemodynamic variables. Hemodynamic variables—(A) d*P*/d*t* max, (B) d*P*/d*t* min, (C) heart rate, and (D) mean arterial pressure—are expressed as means with standard deviations. All values decreased in a dose-dependent manner as isoflurane concentration increased, and values at a concentration of 3% were significantly lower than those at baseline. $^{*}p < 0.05$ versus ISO 0.5% (baseline).

less accurate index of contractility. ESPVR describes the maximal pressure that can be developed by the ventricle at a given LV volume. It is relatively insensitive to load and, thus, is a better index of LV systolic function. ESPVR was significantly lower at an isoflurane concentration of 3% than at baseline ($p = 0.028$; Fig. 2). These findings indicate that LV systolic function is impaired when the isoflurane concentration is high. Cardiovascular function improved when the anesthetic depth was reduced (data not shown).

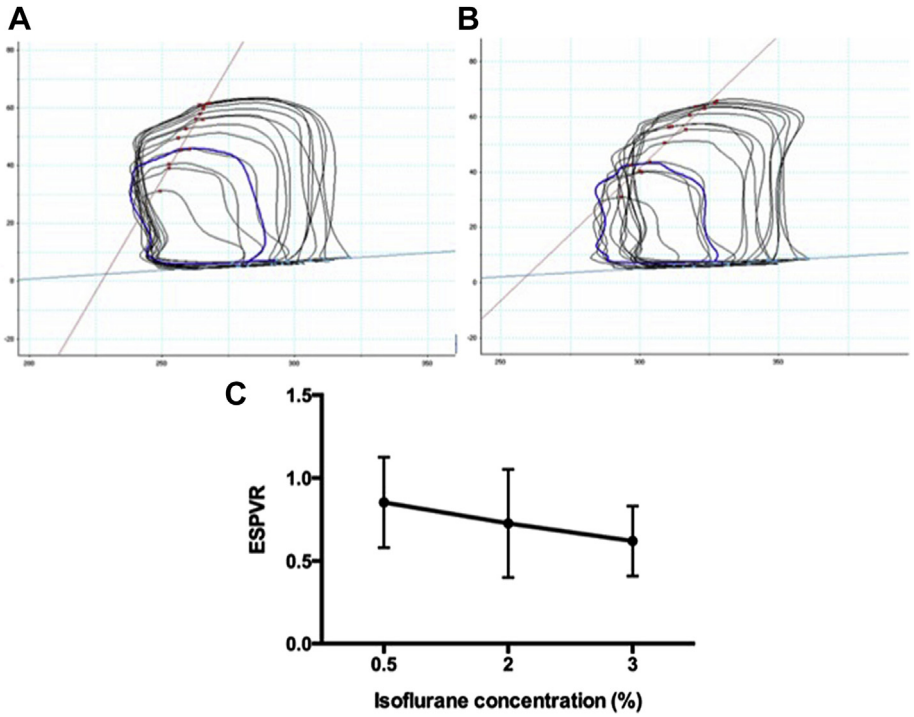

Fig. 2. Change in the pressure–volume loop relation due to change in isoflurane concentration. As isoflurane concentration increased from (A) 1.5% to (B) 2.5%, the pressure–volume loop shifted, and the end-systolic pressure–volume relation (ESPVR) decreased. As compared with the ESPVR at (C) other isoflurane concentrations, the value was significantly lower at a concentration of 3%. ESPVR = end-systolic pressure–volume relation.

3.4. Cardiac diastolic function

d*P*/d*t* min is valuable for evaluating LV diastolic function and progressively decreased as isoflurane concentration increased. It was significantly lower at a concentration of 3% than at baseline. By contrast, LV end-diastolic pressure was not significantly related to isoflurane concentration ($p = 0.298$). These results suggest that isoflurane impairs both LV systolic function and LV diastolic function. However, diastolic impairment was not reflected by LV end-diastolic pressure.

4. Discussion

Our results show that isoflurane has dose-dependent effects on cardiac hemodynamics. At a high dose, inhalational isoflurane decreased heart rate, blood pressure, and myocardial contractility. Previous studies found that although isoflurane decreases peripheral vascular resistance, it has no effect on myocardial contractility [3,10].

There are several possible reasons why high-dose isoflurane impairs myocardial contractility. First, through its hypotensive effects, inhalational isoflurane might indirectly suppress the myocardium. LV end-diastolic pressure was not significantly elevated despite the effect that maximal-concentration isoflurane had on blood pressure. These findings suggest that the peripheral vasodilator effect of maximum-concentration isoflurane decreased venous return from the peripheral circulation. However, contractility could be still impaired because coronary autoregulation would still be compromised when the mean arterial pressure dropped to a low level during high-dose isoflurane inhalation. Coronary blood flow is pressure-dependent, and severe hypotension causes coronary insufficiency, leading to myocardial ischemia and impaired myocardial contractility.

Second, hemodynamic changes at different isoflurane concentrations could be affected by body fluid status, for example, hypovolemic or euvolemic states. In a previous study, at similar anesthesia concentrations, fluid support lessened the hypotensive effect of inhaled isoflurane in dehydrated animals [11]. However, another study found that rapid rehydration did not reverse isoflurane-induced hypotension in normovolemic animals [12]. Therefore, dehydrated animals may be more vulnerable to the effects of anesthetics. It is difficult to measure volume status in rats in hemodynamic studies. In the present experiment, we did not routinely provide fluid supplementation during the entire procedure. Thus, the rats might have been dehydrated, and the hypotensive effect of isoflurane may have been exaggerated. However, ESPVR, an independent preload variable, showed that myocardial contractility decreased with hypotension during high-dose isoflurane inhalation.

Third, aging might be another important factor that affects cardiac hemodynamics at different isoflurane concentrations. Previous studies showed that aging altered cardiac hemodynamics and function in many ways [13]. As compared with young rats, old rats have a lower d*P*/d*t* max, ejection fraction, and cardiac index, and higher LV end-diastolic volume and pressure, at the same anesthesia concentration [13]. The rats we used in our experiments were much older than were those used in a previous study [3]. However, it may be better to use old rats in such studies as hemodynamic characteristics are more likely to be a clinical concern for older adult humans. In any case, a fundamental understanding of hemodynamic effects in elderly adults is crucial.

Our study has several limitations. First, coronary flow was not measured in our experiment. DBP-LV end-diastolic pressure is an indirect measure of coronary perfusion pressure. An isolated heart perfusion model may be needed in order to study coronary physiology and its relationships to inhalational isoflurane. Second, Sprague–Dawley rats are outbred and could thus have heterogeneous responses to inhalational isoflurane. Third, to further examine the effects of isoflurane on peripheral vascular resistance and myocardial contractility, a β antagonist and an α1 antagonist should be used. Fourth, other physiological variables, including temperature, pH, and lactate level, could influence blood pressure but were not examined in our study.

The present results indicate that administration of high-dose isoflurane causes unfavorable hemodynamics changes, and old age and dehydration may predispose animals to these undesirable effects. A better understanding of the hemodynamic effects of isoflurane anesthesia in aged, volume-depleted humans will help clinicians to avoid the use of unnecessarily high doses of isoflurane. Future research is necessary to identify the optimal dose of isoflurane for a preconditioning effect in ischemia–reperfusion injury.

In conclusion, high-dose inhalational isoflurane can affect blood pressure, heart rate, and LV systolic function unfavorably. Careful monitoring of hemodynamics in patients is indicated when higher doses of isoflurane are given. The dose-dependent effect of inhalational isoflurane anesthesia on cardiac hemodynamics should be further examined.

References

[1] Huneke R, Fassl J, Rossaint R, Luckhoff A. Effects of volatile anesthetics on cardiac ion channels. Acta Anaesthesiol Scand 2004;48:547–61.
[2] Stachnik J. Inhaled anesthetic agents. Am J Health Syst Pharm 2006;63:623–34.
[3] Reddy AK, Namiranian K, Lloyd EE, Bryan RM, Taffet GE, Hartley CJ. Effect of isoflurane on aortic impedance in mice. Conf Proc IEEE Eng Med Biol Soc 2009;2009:1104–5.
[4] Merin RG. Physiology, pathophysiology and pharmacology of the coronary circulation with particular emphasis on anesthetics. Anaesthesiol Reanim 1992;17:5–26.
[5] Kato R, Foex P. Myocardial protection by anesthetic agents against ischemia–reperfusion injury: an update for anesthesiologists. Can J Anaesth 2002;49:777–91.
[6] Raphael J, Rivo J, Gozal Y. Isoflurane-induced myocardial preconditioning is dependent on phosphatidylinositol-3-kinase/Akt signalling. Br J Anaesth 2005;95:756–63.
[7] Kiani A, Mirmohammad Sadeghi M, Gharipour M, Farahmand N, Hoveida L. Preconditioning by isoflurane as a volatile anesthetic in elective coronary artery bypass surgery. ARYA Atheroscler 2013;9:192–7.
[8] Suga H. Left ventricular time-varying pressure–volume ratio in systole as an index of myocardial inotropism. Jpn Heart J 1971;12:153–60.
[9] Radovits T, Korkmaz S, Loganathan S, Barnucz E, Bomicke T, Arif R, et al. Comparative investigation of the left ventricular pressure–volume relationship in rat models of type 1 and type 2 diabetes mellitus. Am J Physiol Heart Circ Physiol 2009;297:H125–33.
[10] Riha H, Papousek F, Neckar J, Pirk J, Ostadal B. Effects of isoflurane concentration on basic echocardiographic parameters of the left ventricle in rats. Physiol Res 2012;61:419–23.
[11] Zuurbier CJ, Emons VM, Ince C. Hemodynamics of anesthetized ventilated mouse models: aspects of anesthetics, fluid support, and strain. Am J Physiol Heart Circ Physiol 2002;282:H2099–105.
[12] Valverde A, Gianotti G, Rioja-Garcia E, Hathway A. Effects of high-volume, rapid-fluid therapy on cardiovascular function and hematological values during isoflurane-induced hypotension in healthy dogs. Can J Vet Res 2012;76:99–108.
[13] Pacher P, Mabley JG, Liaudet L, Evgenov OV, Marton A, Hasko G, et al. Left ventricular pressure–volume relationship in a rat model of advanced aging-associated heart failure. Am J Physiol Heart Circ Physiol 2004;287:H2132–7.